Proceedings of the Iowa Academy of Science

Volume 90 | Number

Article 8

1983

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Proc. Iowa Acad. Sci. 90(2): 78-84, 1983

Phloem of Primitive Angiosperms. II. P-Protein in Selected Species of the Ranalean Complex

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A survey of the phloem-protein (P-protein) in species of "primitive" angiosperms was undertaken to provide possible evidence for P-protein function from a phylogenetic point of view. The ontogeny and substructure of P-protein in *Liriodendron tulipifera* and *Magnolia soulangeana* are similar to that of more advanced dicot species. In the light of this information the time of P-protein evolution seems to coincide with the development of the angiosperms themselves.

INDEX DESCRIPTORS: Phloem, sieve-element, P-protein, phloem-protein.

One of the distinguishing features of angiospermous sieve elements is the presence of P-protein. Many theories have been put forth to explain its presence, but as yet no single suggestion is accepted by all workers in the field. Some suggestions include: plugging of the sieve elements during wounding (Crafts and Crisp, 1971), a direct function in solute transport (MacRobbie, 1971; Spanner, 1979), or even some role in cellular recognition mechanisms (Sabnis and Hart, 1979).

Part of the problem stems from the uncertainty of the precise location of P-protein components within the lumina and pores of mature sieve elements due to the so-called "surging phenomenon." Recently, attempts have been made to preserve sieve-tube contents *in situ* by rapid freezing (Fisher, 1975; Johnson, 1978), but even this has been shown to have limitations (Johnson, 1978).

Another course of action has been to study the biochemistry of these proteins (Cronshaw, 1975; Kleinig, 1975). This has contributed a great deal of knowledge but has provided no solution.

We decided to approach this problem from a phylogenetic point of view. Our choice is based on two pieces of information. First, P-protein has not been found in any gymnosperms studied but is present in dicots and a number of monocots (Cronshaw, 1975). Second, study by Sabnis and Hart (1979) indicates that the amount of biochemical similarity in the P-protein of plants corresponds to their taxonomic closeness.

In view of these facts, we are studying P-protein in primitive angiosperms to determine whether it represents a precursor form to that found in more advanced species. This might also aid in explaining its function. We selected *Magnolia soulangeana* Soul., *Liriodendron tulipifera* L., and *Degeneria vitiensis* Bailey and Smith because they are representatives of the Magnoliales, thought to be the most primitive order of the so-called "Ranalean complex" of primitive angiosperms (Cronquist, 1968).

MATERIALS AND METHODS

Expanding internodes of *Magnolia soulangeana* and petioles of different ages from *Liriodendron tulipifera* were obtained from trees located on the St. Ambrose campus and at Pine Hill Cemetery, Davenport, Iowa. The *Degeneria* material consisted of shoots taken from a plant growing in the Missouri Botanical Garden in 1975. Specimens were diced into cacodylate-buffered 6% glutaraldehyde. After six hours of fixation, tissues were rinsed in buffer, postfixed in osmium tetroxide, and dehydrated in an ethanol-propylene oxide series. This was followed by embedment in Spurr's resin (Spurr, 1969). A Sorvall MT-1 ultramicrotome was used to take thin sections, some of which were stained with potassium permanganate and lead citrate, whereas others received the typical uranyl acetate-lead citrate sequence. The structure of the P-protein appeared the same in either case, but the former stain combination tended to overstain. Observations of the primary phloem of these species were made with a RCA EMU-3E electron microscope.

OBSERVATIONS

Liriodendron

Initially, P-protein occurs as small masses of tubular material free in the cytoplasm (Figs. 1 and 2). The smallest of such bodies was found to be only 0.7 μm in length. The tubules comprising the P-protein bodies are approximately 19 nm in diameter. In all cases the tubules within a body are oriented roughly parallel to the longitudinal axis of the young sieve element. The tubules appear very flexible; much more so than microtubules, for example. Some tubules appear to consist of tightly wound helices; in others the helices are not evident (Fig. 2). The double arrows in Figure 2 indicate very loosely wound helices on the periphery of the P-protein body.

The young P-protein bodies are "zones of exclusion." That is, other typical cytoplasmic components are absent from the region of tubules. The periphery of the P-protein bodies is consistently associated with ribosomes (Figs. 1, 2, 3) and, to a lesser extent, with dictyosomes and spiny vesicles (Fig. 3).

It is not known how many P-protein bodies develop in a young sieve element of *Liriodendron*. Observations indicate the number is small, but as the sections taken are very thin this impression could be misleading.

Fig. 1-4: ***Liriodendron***

Fig. 1. Portion of a longitudinal view of a thick-walled sieve element. Note young P-protein body (arrow) and compare its size to dictyosome to its left. x28,500.

Fig. 2. Higher magnification of another young P-protein body. Width of tubules may be compared to microtubules (MT) lining wall. Developing body represents a zone of exclusion for other subcellular components. 'A' indicates a tubule which shows no substructure. 'B' indicates a tubule with repeating subunits which may represent a tightly wound helix. Double arrows indicate tubules in process of assembly. x57,200.

Fig. 3. Sieve element at a stage of development even earlier than Figures 1 and 2, as it is thin-walled. At this stage P-protein is present. Arrow indicates a spiny vesicle at periphery of the P-protein body. x38,600.

Fig. 4. An enlarging P-protein body. At this stage some tubules exhibit a curvature so that they are no longer parallel to longitudinal axis of cell. x19,500.

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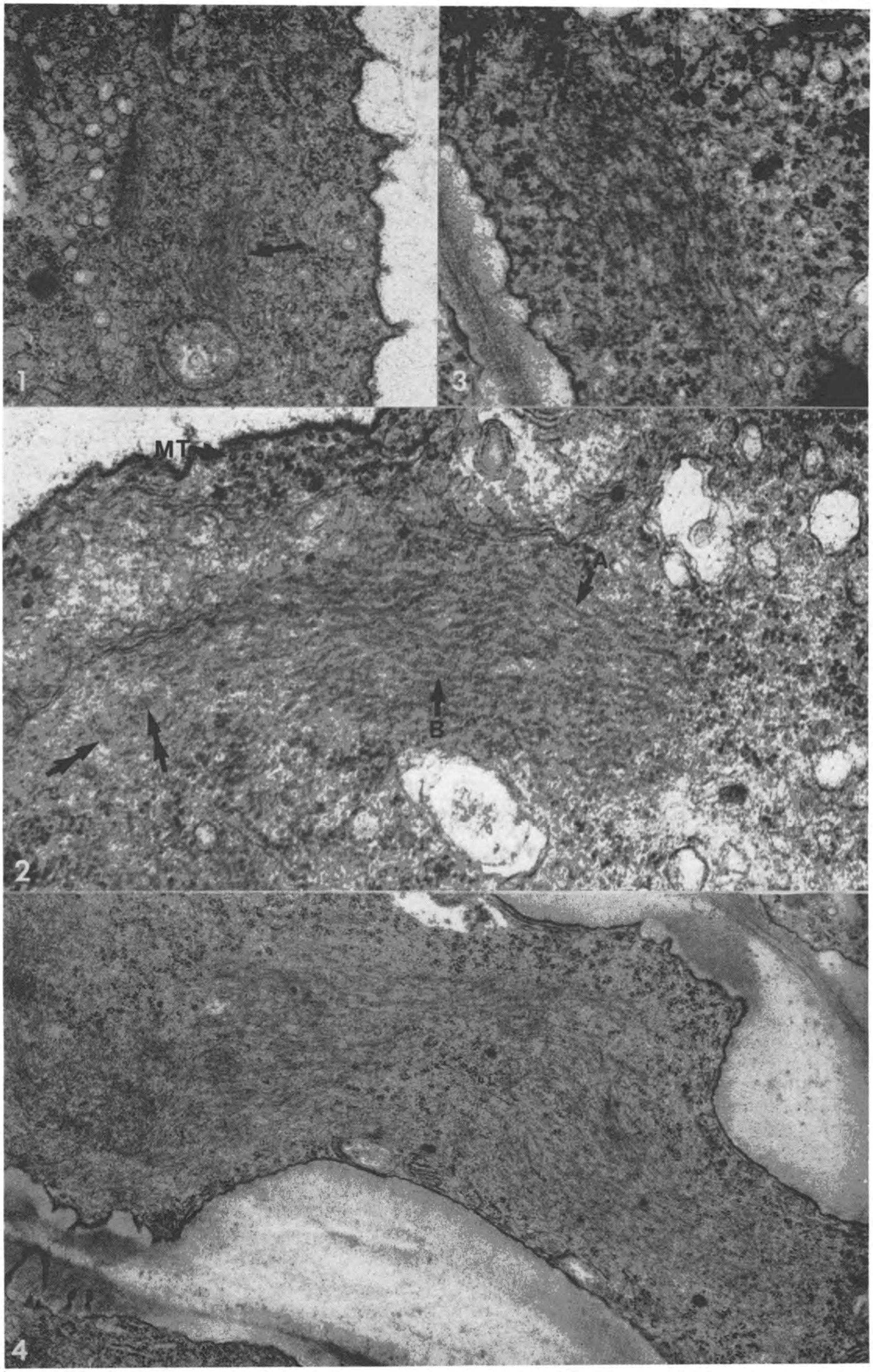

1
3
MT
A
B
2
4

Figures 1 and 2 show very young P-protein bodies during the wall-thickening stage of sieve-element ontogeny. This stage occurs prior to any degradative changes associated with functional sieve elements. One example of a P-protein body was seen even before wall thickening had begun (Fig. 3). Thus the initial presence of P-protein bodies is one of the earliest distinctive features of sieve elements.

As wall thickening continues, the sieve element elongates. The elongation is accompanied by an increase in size of the P-protein body (Fig. 4). The increase is one of width as well as length, and is associated with an increase in the number of tubules. Whether there is an increase in tubule length is uncertain due to the flexible nature of the individual tubules. At this stage, only one large P-protein body is present; thus enlargement and fusion of smaller bodies is suspected. As the P-protein body enlarges, so of course does the zone of exclusion (Fig. 4). As a result, ribosomes, membranes, and organelles are displaced to the periphery of the sieve element.

Enlargement of the P-protein body continues throughout the wall-thickening period until it is the largest object within the cytoplasm. Portions of it are curved and become perpendicular to the longitudinal axis of the cell (Fig. 4).

Cross-sections of the P-protein body at this time show the hollow centers of the tubules (Fig. 5). Measurements show the tubule wall to have a thickness of about 8.5 nm. The tubules present angular outlines with protruding spokes (Fig. 5, double arrow). It has been postulated that these spokes are responsible for keeping the P-protein body intact (Cronshaw, 1975).

The sequence of events of P-protein synthesis in the sieve elements is followed by a selective lytic process which involves nuclear degeneration, general cytoplasmic clearing, and opening of the pores between cells (Dute, 1983). Figures 6 and 7 present the status of the P-protein during early in cytoplasmic lysis. The cytoplasmic background both within and beyond the P-protein body clears. Additionally, the tubules now appear to be separating one from another. In Figures 8 and 9 a later lytic stage is shown. The P-protein tubules are now definitely dispersed, and the P-protein body as such has ceased to exist.

The helical nature of the individual P-protein strands becomes more evident as sieve-element maturity is reached (Fig. 10). The P-protein is now in the so-called fibrillar or striated form. This is thought to be derived from the tubular form *via* stretching of a double helix (rather than a single helix) comprising the tubules (Parthasarathy and Mühlethaler, 1969). Although the diameter of P-protein fibrils varies greatly, the average diameter (16.5-17 nm) is somewhat less than the 19-22 nm diameter for the tubules. The variation in fibril diameter may reflect different degrees of stretching of the tubular form of P-protein. In very old sieve elements (so determined by their location within the vascular bundle) the fibrils appear to unwind into threads of approximately 7.5-8 nm thickness (Fig. 11, arrows).

The location of P-protein within mature sieve elements varies greatly. It has been seen both evenly dispersed throughout the lumen or concentrated in the regions of the sieve plates. This is to be expected since no precautions were taken to minimize surging. Also, in some cases P-protein fibrils aggregate into masses in which the striations are in register (Fig. 12).

The amounts of P-protein per mature sieve element vary from little if any to a great deal. Metaphloem sieve elements seem to possess more of the material than their protophloem counterparts.

Magnolia

P-protein tubules are also of common occurrence in the sieve elements of *Magnolia soulangeana*. They are especially noticeable in sieve elements approaching maturity or in those that are newly-matured. Even after maturity, the tubules (running parallel to the long axis of the cell) are not totally dispersed (Fig. 13). Structurally, they appear similar to those found in *Liriodendron*, with the spokes (as seen in longitudinal views, Fig. 14), a translucent center, angular outline, and a diameter of 19-24 nm. Further, the tightly wound helical subunits are visible even at this stage (Fig. 14, double arrow).

As maturation progresses, the P-protein becomes a mixture of tubules and fibrils of various thicknesses (13-18nm) (Fig. 15). This stage is followed by one in which the P-protein is largely fibrillar (Fig. 16). Very old sieve elements possess a mixture of fibrils and threads, the latter formed by the unwinding of the helices.

Young sieve elements possess P-protein bodies which enlarge with time. As in *Liriodendron*, these bodies are zones of exclusion. In general, the overall development of P-protein in *Magnolia* parallels that of *Liriodendron* except that in the former the tubular form continues well into maturity.

Degeneria

Because of the limited amount of material available (due to inadequate fixation) little information was gathered on *Degeneria*. Like other dicots, however, the sieve elements do possess P-protein, and early in maturity it is present as striated fibrils (Fig. 17, arrow).

DISCUSSION

The three species investigated have been shown to possess P-protein, a feature typical of dicot phloem (Evert, 1977). We have observed this material to have at least two distinct forms — tubular and fibrillar — the latter seemingly derived from the former.

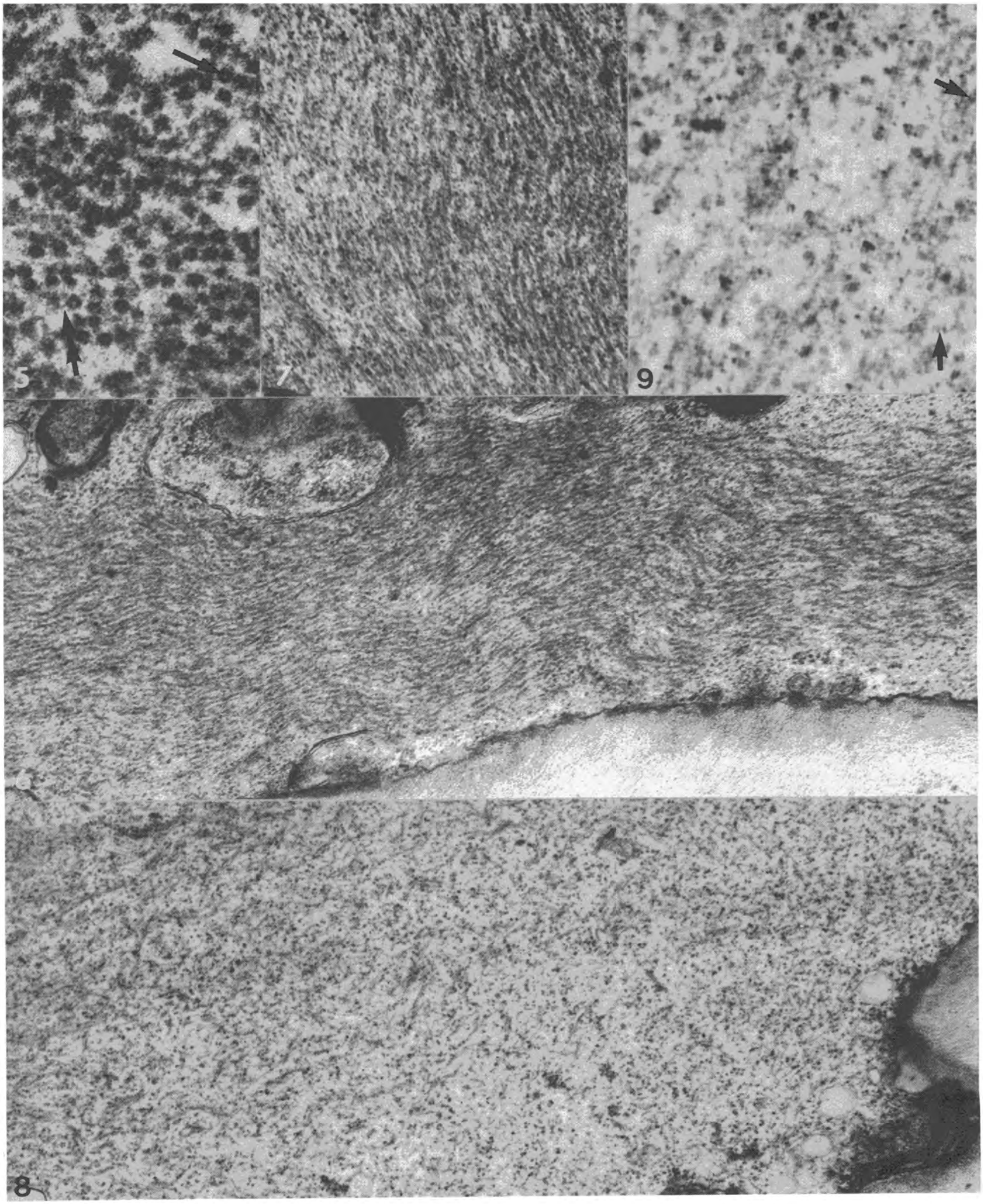
The P-protein ontogeny described for both *Liriodendron* and *Magnolia* appears to parallel that found in *Nicotiana* by Cronshaw and Esau (1967). They reported that "slime" (P-protein) arises as small groups of tubules of 23 nm diameter. The P-protein bodies enlarge and then disaggregate during the lytic phase of sieve-element development. The tubules become reorganized into striated fibrils only 15 nm wide. A similar sequence of events has been found in the sieve elements of *Coleus* (Steer and Newcomb, 1969). Nevertheless, further study has complicated this interpretation a great deal. In *Ricinus* for example, (Cronshaw, 1975), no tubular P-protein exists. *Cucurbita* sieve elements (Cronshaw and Esau, 1968) possess two different types of P-protein bodies, one which initially consists of tubules, the other in which the tubules develop from a fibrillar material. Even later work with tobacco (Cronshaw, 1975) has shown the presence of a finely granular P-protein associated with the endoplasmic reticulum of young sieve elements. Esau (1971) found that in *Mimosa*, fine filaments become organized into P-protein tubules in very young sieve elements. In the earliest stages of development observed in the present study, P-protein appeared as tubules. It was noted however, that loosely wound helices occurred at the periphery of small P-protein bodies (Fig. 2). These may be individual tubules in the process of assembly. Thus, the possibility exists for a yet earlier stage of development.

Fig. 5-9: *Liriodendron*

Fig. 5. Portion of P-protein body in cross-section in a sieve element at height of wall thickening. Hollow nature of tubules is apparent (arrow). Also, tubules appear to be connected by spokelike structures (double arrow). x99,500.

Fig. 6 and 7. A P-protein body early in cell lysis. Separation of tubules has begun. x32,600; 56,300.

Fig. 8 and 9. Dispersed P-protein later in cell lysis. Note that with the clearing of background, tight helical nature of elongate strands can be clearly seen (arrows). Tubular nature of the individual P-protein units is less evident. x22,700; 77,400



5
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9
6
8

The conformational change from tubule to striated fibril has been noted by many authors (q.v. Cronshaw, 1975). It is thought that the tubules consist of helical strands (probably two), which upon distention (stretching) produce the striated structures known as fibrils (Parthasarathy and Mühlethaler, 1969; Cronshaw, Gilder, and Stone, 1973). Evidence for stretching comes from *Nicotiana* (Parthasarathy and Mühlethaler, 1969); as the tubules decrease in diameter the distance between the cross striations of the helices increases. We found the same to be true in *Magnolia*.

Recently, Lawton and Newman (1979) have provided evidence for the artifactual nature of fibrillar P-protein. They suggested that the tubular form remains as such in the mature sieve element, and that striated fibrils form as a result of turgor pressure release during the process of dicing prior to fixation. The presence of P-protein tubules well into maturity in some *Magnolia* sieve elements seems to provide evidence for their contention.

Thus the substructure and ontogeny of the P-protein in *Magnolia*, *Liriodendron*, and to the extent it was observed in *Degeneria*, are similar to that of more advanced dicots. If, in fact, the Ranalean complex represents angiosperms that have retained primitive traits, then perhaps P-protein evolved and became fixed in its present form very early in the development of flowering plants.

ACKNOWLEDGEMENTS

The authors express their gratitude to Dr. J. E. Mikesell, Gettysburg College, for his critical reading of the manuscript, *Degeneria* material was obtained through the kind assistance of S. E. Eichhorn, University of Wisconsin.

Fig. 10-15: ***Liriodendron*** (10-12); ***Magnolia*** (13-15).

Fig. 10. P-protein in a newly-matured sieve element. Elongate elements are now slightly striated. Widths vary from fibrillar to tubular. x79,800.

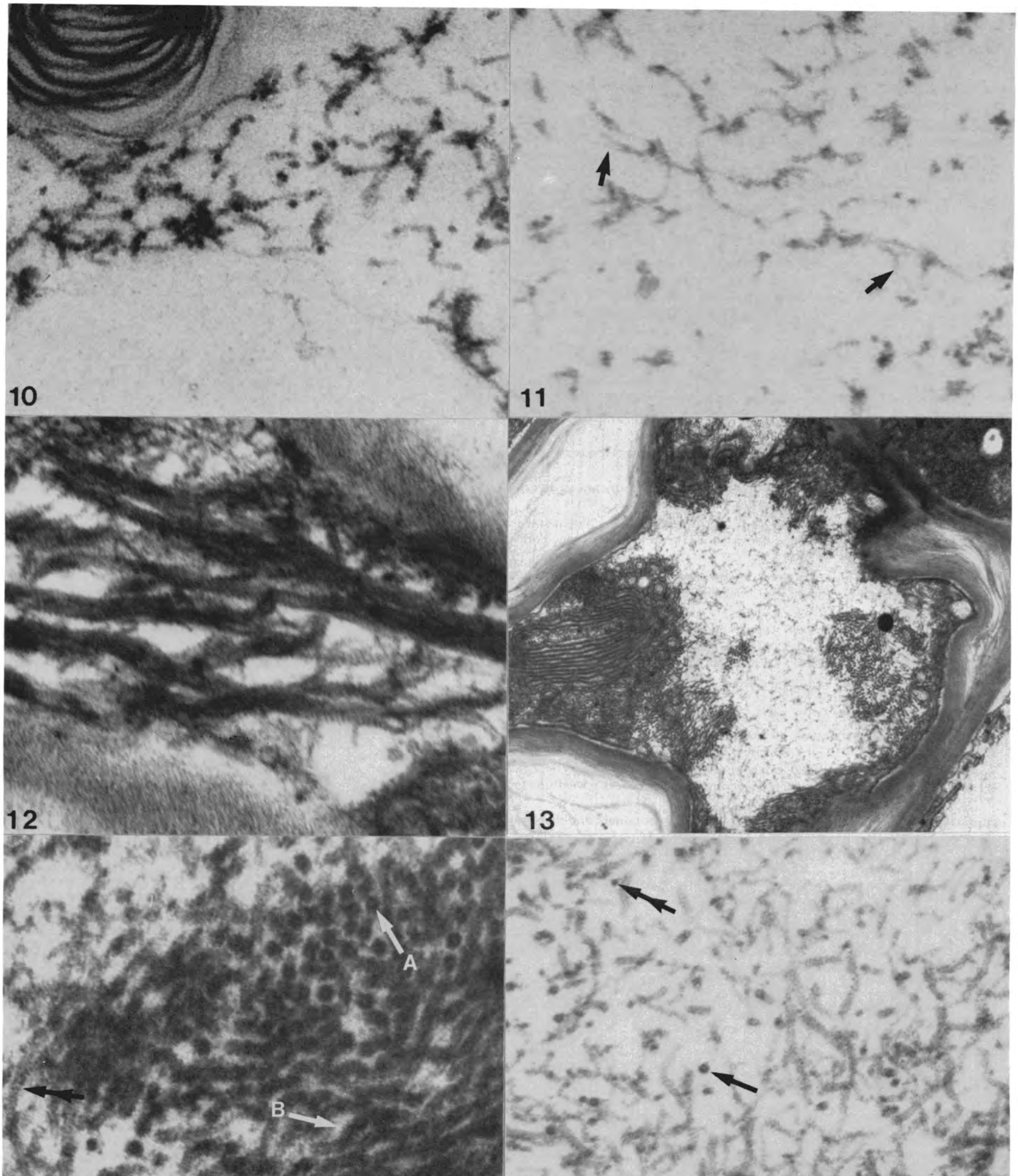
Fig. 11. An aged sieve element with P-protein that is largely fibrillar. In a couple cases (arrows) fibrils appear to be unwinding. x76,400.

Fig. 12. Masses of fibrillar P-protein in a mature sieve element which are aggregated so that striations are in register. x69,800.

Fig. 13. Sieve element at maturity (note clear hyaloplasm), in which tubular P-protein, still partially aggregated, is present. x15,300.

Fig. 14. High magnification of Fig. 13 showing the substructure of tubules. 'A' shows lumen; double arrow shows tightly-wound helical nature of tubule; 'B' indicates spokes of a tubule in longitudinal section. x115,900.

Fig. 15. Mixture of a few fibrils with tubules in a mature element. Arrows show distinction between tubules (single arrow) and fibrils (double arrow) in cross-section. x77,000.


10
11
12
13
A
B
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15

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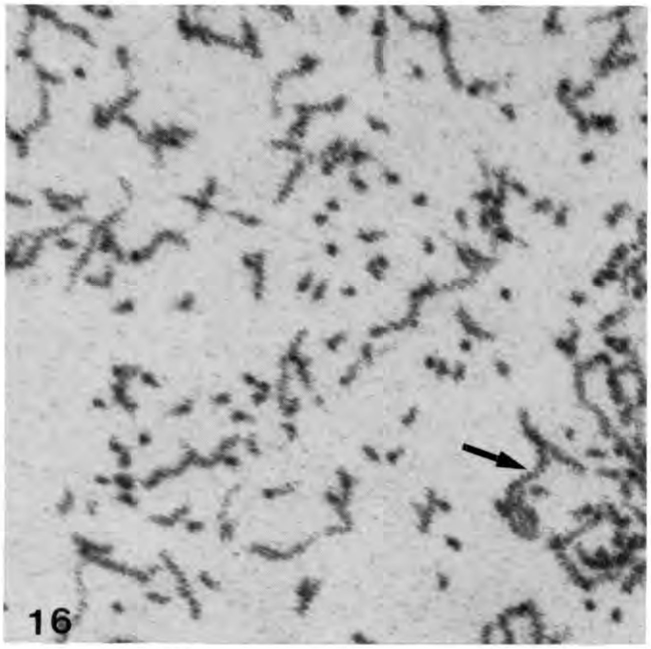
Fig. 16-17: ***Magnolia*** **(16);** ***Degeneria*** **(17).**



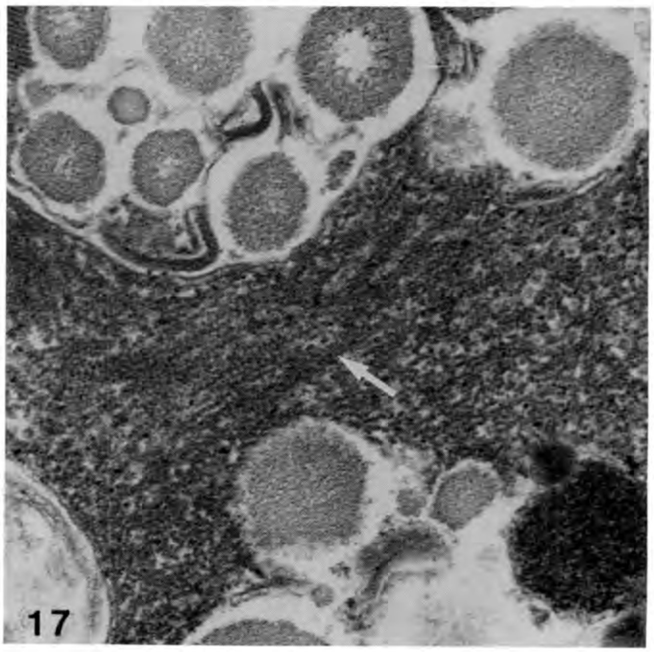


Fig. 16. A sieve element older than that in Fig. 15 in which fibrillar or striated form is dominant. Arrow indicates a fibril in which double helix is quite evident. x74,000.

Fig. 17. Striated fibrils in a *Degeneria* sieve element (arrow). x75,000.